

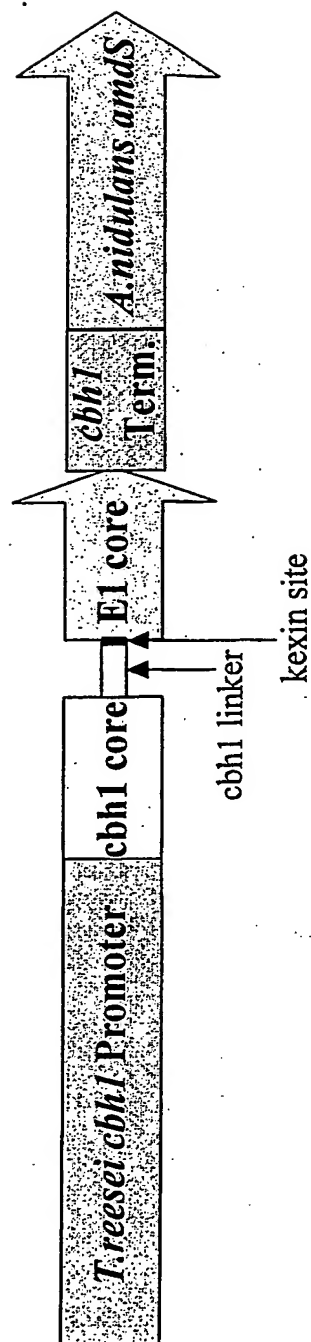
FIGURE 1

# CBH1-E1 Fusion Construct

*T. reesei cbh1* core, linker (no CBD)

+

*Acidothrmus cellulolyticus endoglucanase 1* core (E1)



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## Figure 2

DNA sequence of *T.reesei cbh1* signal sequence+catalytic domain+linker (1570 bases)

ATGTATCGGAAGTTGGCCGTCATCTCGGCCTTCTTGGCCACAGCTCGTGCTCA  
GTCGGCCTGCACTCTCCAATCGGAGACTCACCCGCCTCTGACATGGCAG  
AAATGCTCGTCTGGTGGCACTTGCACCTAACAGACAGGCTCCGTGGTCA  
TCGACGCCAACTGGCGCTGGACTCACGCTACGAACAGCAGCACGAACTG  
CTACGATGGCAACACTTGGAGCTCGACCCTATGTCCTGACAACGAGACC  
TGCGCGAAGAAGTGTGTCTGGACGGTGCCGCCTACGCGTCCACGTACG  
GAGTTACCACGAGCGGTAACAGCCTCTCCATTGGCTTTGTCACCCAGTC  
TGCGCAGAAGAACGTTGGCGCTCGCCTTTACCTTATGGCGAGCGACACG  
ACCTACCAGGAATTCACCCTGCTTGGCAACGAGTTCTCTTTCGATGTTGA  
TGTTTCGCAGCTGCCGTAAGTGACTTACCATGAACCCCTGACGTATCTTC  
TTGTGGGCTCCCAGCTGACTGGCCAATTTAAGGTGCGGCTTGAACGGAG  
CTCTCTACTTCGTGTCCATGGACGCGGATGGTGGCGTGAGCAAGTATCC  
CACCAACACCGCTGGCGCCAAGTACGGCACGGGGTACTGTGACAGCCAG  
TGTCCTCCGCGATCTGAAGTTCATCAATGGCCAGGCCAACGTTGAGGGCT  
GGGAGCCGTCATCCAACAACGCAAACACGGGCATTGGAGGACACGGAA  
GCTGCTGCTCTGAGATGGATATCTGGGAGGCCAACTCCATCTCCGAGGC  
TCTTACCCCCCACCCTTGCACGACTGTGCGCCAGGAGATCTGCGAGGGT  
GATGGGTGCGGCGGAACTTACTCCGATAACAGATATGGCGGCACTTGCG  
ATCCCGATGGCTGCGACTGGAACCCATACCGCCTGGGCAACACCAGCTT  
CTACGGCCCTGGCTCAAGCTTTACCCTCGATACCACCAAGAAATTGACC  
GTTGTACCCAGTTCGAGACGTCGGGTGCCATCAACCGATACTATGTCC  
AGAATGGCGTCACTTTCCAGCAGCCCAACGCCGAGCTTGGTAGTTACTC  
TGGCAACGAGCTCAACGATGATTACTGCACAGCTGAGGAGGCAGAATTC  
GGCGGATCCTCTTTCTCAGACAAGGGCGGCCTGACTCAGTTCAAGAAGG  
CTACCTCTGGCGGCATGGTTCTGGTCATGAGTCTGTGGGATGATGTGAG  
TTTGATGGACAAACATGCGCGTTGACAAAGAGTCAAGCAGCTGACTGAG  
ATGTTACAGTACTACGCCAACATGCTGTGGCTGGACTCCACCTACCCGA  
CAAACGAGACCTCCTCCACACCCGGTGCCGTGCGCGGAAGCTGCTCCAC  
CAGCTCCGGTGTCCCTGCTCAGGTCGAATCTCAGTCTCCCAACGCCAAG  
GTCACCTTCTCCAACATCAAGTTCGGACCCATTGGCAGCACCGGCAACC  
CTAGCGGCGGCAACCCCTCCCGGCGGAAACCCGCCTGGCACCACCACCCCG  
CCGCCCAGCCACTACCACTGGAAGCTCTCCCGGACCTACTAGT

## Figure 3

Amino acid sequence of *T.reesei cbh1* signal sequence + catalytic domain + linker (480 amino acids)

MYRKLAVISAFLATARAQSACTLQSETHPPLTWQKCSSGGTCTQQTGSVVID  
ANWRWTHATNSSTNCYDGNTWSSTLCPDNETCAKNCCLDGAAYASTYGVT  
TSGNSLSIGFVTQSAQKNVGARLYLMASDTTYQEFTLLGNEFSFDVDVSQLP  
CGLNGALYFVSMADADGGVSKYPTNTAGAKYGTGYCDSQCPRDLKFINGQA  
NVEGWEPSSNNANTGIGGHGSCCSEMDIWEANSISEALTPHPCTTVGQEICE  
GDGCGGTYSNRYGGTCDPDGCDWNPYRLGNTSFYGPSSFTLDTTKKLT  
VVTQFETSGAINRYVQNGVTFQQPNAELGSYSGNELNDDYCTAEEAEFGG  
SSFSDKGGLTQFKKATSGGMVLVMSLWDDYYANMLWLDSTYPTNETSSTP  
GAVRGSCSTSSGVPAQVESQSPNAKVTFESNIKFGPIGSTGNPSGGNPPGGNPPG  
TTTTRRPATTTGSSPGPTS

## Figure 4

DNA sequence of *Acidothermus cellulolyticus* GH5A endoglucanase 1 catalytic domain (1077 bases)

GCGGGCGGCGGCTATTGGCACACGAGCGGCCGGGAGATCCTGGACGCGAAC  
AACGTGCCGGTACGGATCGCCGGCATCAACTGGTTTGGGTTCGAAACCTGCA  
ATTACGTTCGTGCACGGTCTCTGGTCACGCGACTACCGCAGCATGCTCGACCA  
GATAAAGTCGCTCGGCTACAACACAATCCGGCTGCCGTACTCTGACGACATT  
CTCAAGCCGGGCACCATGCCGAACAGCATCAATTTTACCAGATGAATCAGG  
ACCTGCAGGGTCTGACGTCTTGCAGGTCATGGACAAAATCGTCGCGTACGC  
CGGTACAGATCGGCCTGCGCATCATTCTTGACCGCCACCGACCGGATTGCAGC  
GGGCAGTCGGCGCTGTGGTACACGAGCAGCGTCTCGGAGGCTACGTGGATTT  
CCGACCTGCAAGCGCTGGCGCAGCGCTACAAGGGAAACCCGACGGTCGTCTG  
GCTTTGACTTGACACAACGAGCCGCATGACCCGGCCTGCTGGGGCTGCGGCGA  
TCCGAGCATCGACTGGCGATTGGCCGCCGAGCGGGCCGGAAACGCCGTGCTC  
TCGGTGAATCCGAACCTGCTCATTTCGTCGAAGGTGTGCAGAGCTACAACG  
GAGACTCCTACTGGTGGGGCGGCAACCTGCAAGGAGCCGGCCAGTACCCGGT  
CGTGCTGAACGTGCCGAACCGCCTGGTGTACTCGGCGCACGACTACGCGACG  
AGCGTCTACCCGCAGACGTGGTTCAGCGATCCGACCTTCCCCAACAACATGC  
CCGGCATCTGGAACAAGAAGTGGGGATACCTCTTCAATCAGAACATTGCACC  
GGTATGGCTGGGCGAATTCGGTACGACACTGCAATCCACGACCGACCGAGACG  
TGGCTGAAGACGCTCGTCCAGTACCTACGGCCGACCGCGCAATACGGTGCGG  
ACAGCTTCCAGTGGACCTTCTGGTCCTGGAACCCCGATTCCGGCGACACAGG  
AGGAATTCTCAAGGATGACTGGCAGACGGTCGACACAGTAAAAGACGGCTAT  
CTCGCGCCGATCAAGTCGTCGATTTTCGATCCTGTCTCGGC

**Figure 5**

Amino acid sequence of *Acidothermus cellulolyticus* GH5A endoglucanase 1 catalytic domain (359 amino acids)

AGGGYWHTSGREILDANNVPVRIAGINWFGFETCNYYVHGLWSRDYRSMLDQI  
KSLGYNTIRLPYSDDILKPGTMPNSINFYQMNQDLQGLTSLQVMDKIVAYAGQIG  
LRILDRHRPDCSGQSALWYTSSVSEATWISDLQALAQRKYKGNPTVVGFDLHNEP  
HDPACWGCGDPSIDWRLAAERAGNAVLSVNPNNLIFVEGVQSYNGDSYWWGG  
NLQGAGQYPVVLNVPNRLVYSAHDYATSVYPQTWFS DPTFPNNMPGIWNKNW  
GYLFNQNIAPVWLGEFGTTLQSTTDQTLVQYLRPTAQYGADSFQWTFWS  
WNPDSGDTGGILKDDWQTVDTVKDGYLAPIKSSIFDPVG

## FIGURE 6

DNA sequence of *Acidothermus cellulolyticus* GH48 cellulase catalytic domain (1914 bases)

AACGACCCGTACATCCAGCGGTTCTCACGATGTACAACAAGATTCACGACC  
CAGCGAACGGCTACTTCAGCCCCGACGGGAATTCCTACCACTCGGTAGAAAC  
GCTCATCGTTGAGGCACCGGACTACGGGCACGAGACAACCTTCGGAGGCGTAC  
AGCTTCTGGCTCTGGCTCGAAGCGACGTACGGCGCAGTGACCGGCAACTGGA  
CGCCGTTCAACAACGCCTGGACGACGATGGAAACGTACATGATCCCGCAGCA  
CGCGGACCAGCCGAACAACGCGTCGTACAACCCCAACAGCCCGGCGTCGTAC  
GCTCCGGAAGAGCCGCTGCCCAGCATGTACCCGGTTGCCATCGACAGCAGCG  
TGCCGGTTGGGCACGACCCGCTCGCCGCCGAATTGCAGTCGACGTACGGCAC  
TCCGGACATTTACGGCATGCACTGGCTGGCCGACGTTGACAACATCTACGGA  
TACGGCGACAGCCCCGGCGGTGGTTGCGAACTCGGTCCTTCCGCTAAGGGCG  
TCTCCTACATCAACACATTCCAGCGCGGCTCGCAGGAGTCCGTCTGGGAGAC  
GGTACCCAGCCGACGTGCGACAACGGCAAGTACGGTGGGGCGCACGGCTA  
CGTCGACCTGTTTCATCCAGGGTTTCGACGCCGCCGACGTGGAAGTACACCGAT  
GCCCCGGACGCCGACGCCCGTGCCGTCCAGGCTGCGTACTGGGCCTACACCT  
GGGCATCGGCGCAGGGCAAGGCAAGCGCGATTGCCCCGACGATCGCCAAGG  
CGGCCAAACtCGGCGACTACCTGCGGTACTCGCTCTTTGACAAGTACTTCAAG  
CAGGTCGGCAACTGCTACCCGGCCAGCTCCTGCCCTGGAGCAACCGGACGCC  
AGAGCGAGACCTACCTGATCGGCTGGTACTACGCCTGGGGCGGCTCAAGCCA  
AGGCTGGGCCTGGCGCATTGGTGACGGCGCCGCGCACTTCGGCTACCAGAAT  
CCGCTTGCCGCGTGGGCGATGTGCAACGTGACACCGCTCATTCCGCTCTCGCC  
CACGGCAAAGAGCGACTGGGCGGCGAGCTTGCAGCGCCAGCTGGAGTTCTAC  
CAGTGGTTGCAATCCGCGGAAGGAGCCATTGCGGGCGGCGCCACCAACAGCT  
GGAACGGCAATTACGGGACCCCGCCGGCCGGAGACTCGACCTTCTACGGCAT  
GGCGTACGACTGGGAGCCGGTCTACCACGACCCGCCGAGCAACAACCTGGTTC  
GGCTTCCAGGCGTGGTCCATGGAACGGGTTGCCGAGTACTACTACGTCACCG  
GCGACCCGAAGGCCAAGGCGCTGCTCGACAAGTGGGTCGCATGGGTGAAGC  
CGAATGTCACCACCGGTGCCTCATGGTTCGATTCCGTCGAATTTGTCCTGGAGC  
GGCCAACCGGATACCTGGAATCCGAGCAACCCAGGAACGAATGCCAACCTG  
CACGTGACCATCACGTCGTCCGGGCAGGACGTCCGGTGTGCCGCGGCGCTCG  
CGAAGACACTCGAGTACTACGCGGCAAAATCCGGCGATACGGCCTCGCGCGA  
CCTCGCGAAGGGATTGCTCGACTCCATGTGGAACAACGACCAGGACAGCCTC  
GGTGTGAGCACACCGGAGACGCGGACCGACTACTCTCGGTTCACTCAGGTGT  
ACGACCCGACGACTGGTGACGGCCTCTACATCCCGTCGGGTTGGACGGGGAC  
CATGCCCAACGGTGACCAAATCAAGCCGGGTGCGACCTTCCTGAGCATCCGG  
TCCTGGTACACCAAGGATCCGCAAGTGGTTCGAAGGTGCAGGCGTACCTCAACG  
GCGGGCCTGCTCCGACGTTCAACTACCACCGGTTCTGGGCGGAGTCCGACTT  
CGCGATGGCGAACGCCGATTTTGGCATGCTCTTCCCATCCGGG

## FIGURE 7

Amino acid sequence of *Acidothermus cellulolyticus* 48 catalytic domain (638 amino acids)

NDPYIQRFLTMYNKIHPANGYFSPQGIPYHSVETLIVEAPDYGHETTSEAYSW  
LWLEATYGAVTGNWTPFNNAWTTMETYMIPQHADQPNNASYNPNSPASYAPEE  
PLPSMYPVAIDSSVPVGHDPAAELQSTYGTPDIYGMHWLADVNDNIYGYGDS  
GGCELGPSAKGVSYINTFQRGSQESVWETVTQPTCDNGKYGGAHGYVDLFIQGS  
TPPWKYTDAPDADARAVQAAYWAYTWASAQGKASAIPTIAKAAKLGDYLR  
YSLFDKYFKQVGNCYPASSCPGATGRQSETYLIGWYYAWGGSSQGWAWRIGD  
GAAHFGYQNPLAAWAMSNVTPLIPLSPTAKSDWAASLQRQLEFYQWLQSAEGA  
IAGGATNSWNGNYGTPPAGDSTFYGMAYDWEVPVYHDPPSNNWFGFQAWSMER  
VAEYYYVTGDPKAKALLDKWVAWVKPNVTTGASWSIPSNLSWSGQPDTWNPS  
NPGTNANLHVTITSSGQDVGVAAALAKTLEYAAKSGDTASRDLAKGLLDSMW  
NNDQDSLGVSTPETRTDYSRFTQVYDPTTGDGLYIPSGWTGTMPNGDQIKPGAT  
FLSIRSWYTKDPQWSKVQAYLNGGPAPTFNYHRFWAESDFAMANADFGMLFPS  
G

## FIGURE 8A

DNA sequence of *Acidothermus cellulolyticus* GH74 catalytic domain

GCGACGACTCAGCCGTACACCTGGAGCAACGTGGCGATCGGGGGCGGCGGC  
TTTGTGACGCGGATCGTCTTCAATGAAGGTGCACCGGGAATTCTGTACGTGCG  
GACGGACATCGGGGGGATGTATCGATGGGATGCCGCCAACGGGCGGTGGAT  
CCCTCTTCTGGATTGGGTGGGATGGAACAATTGGGGGTACAACGGCGTCGTC  
AGCATTGCGGCAGACCCGATCAATACTAACAAGGTATGGGCCGCCGTCGGAA  
TGTACACCAACAGCTGGGACCCAAACGACGGAGCGATTCTCCGCTCGTCTGA  
TCAGGGCGCAACGTGGCAAATAACGCCCCCTGCCGTTCAAGCTTGGCGGCAAC  
ATGCCCCGGGCGTGGAATGGGCGAGCGGCTTGCGGTGGATCCAAACAATGACA  
ACATTCTGTATTTTCGGCGCCCCGAGCGGCAAAGGGCTCTGGAGAAGCACAGA  
TTCCGGCGCGACCTGGTCCCAGATGACGAACTTTCCGGACGTAGGCACGTAC  
ATTGCAAATCCCACTGACACGACCGGCTATCAGAGCGATATTCAAGGCGTCG  
TCTGGGTCGCTTTCGACAAGTCTTCGTCATCGCTCGGGCAAGCGAGTAAGACC  
ATTTTTGTGGGCGTGCGGATCCCAATAATCCGGTCTTCTGGAGCAGAGACG  
GCGGCGCGACGTGGCAGGCGGTGCCGGGTGCGCCGACCGGCTTCATCCCGCA  
CAAGGGCGTCTTTGACCCGGTCAACCACGTGCTCTATATTGCCACCAGCAAT  
ACGGGTGGTCCGTATGACGGGAGCTCCGGCGACGTCTGGAAATTCTCGGTGA  
CCTCCGGGACATGGACGCGAATCAGCCCGGTACCTTCGACGGACACGGCCAA  
CGACTACTTTGGTTACAGCGGCCTCACTATCGACCGCCAGCACCCGAACACG  
ATAATGGTGGCAACCCAGATATCGTGGTGGCCGGACACCATAATCTTTCGGA  
GCACCGACGGCGGTGCGACGTGGACGCGGATCTGGGATTGGACGAGTTATCC  
CAATCGAAGCTTGCGATATGTGCTTGACATTTTCGGCGGAGCCTTGGCTGACCT  
TCGGCGTACAGCCGAATCCTCCCGTACCGAGTCCGAAGCTCGGCTGGATGGA  
TGAAGCGATGGCAATCGATCCGTTCAACTCTGATCGGATGCTCTACGGAACA  
GGCGCGACGTTGTACGCAACAAATGATCTCACGAAGTGGGACTCCGGCGGCC  
AGATTCATATCGCGCCGATGGTCAAAGGATTGGAGGAGACGGCGGTAAACG  
ATCTCATCAGCCCGCGTCTGGCGCCCCGCTCATCAGCGCTCTCGGAGACCTC  
GGCGGCTTCACCCACGCCGACGTTACTGCCGTGCCATCGACGATCTTCACGTC



## FIGURE 8B

ACCGGTGTTACGACCGGCACCAGCGTCGACTATGCGGAATTGAATCCGTCG  
ATCATCGTTCGCGCTGGAAGTTTCGATCCATCGAGCCAACCGAACGACAGGC  
ACGTCGCGTTCTCGACAGACGGCGGCAAGAACTGGTTCCAAGGCAGCGAACC  
TGGCGGGGTGACGACGGGCGGCACCGTCGCCGCATCGGCCGACGGCTCTCGT  
TTCGTCTGGGCTCCCGGCGATCCCGGTACGCCTGTGGTGTACGCAGTCGGATT  
TGGCAACTCCTGGGCTGCTTCGCAAGGTGTTCCCGCCAATGCCCAGATCCGCT  
CAGACCGGGTGAATCCAAAGACTTTCTATGCCCTATCCAATGGAACCTTCTAT  
CGAAGCACGGACGGCGGCGTGACATTCCAACCGGTCGCGGCCGGTCTTCCGA  
GCAGCGGTGCCGTCGGTGTTCATGTTCCACGCGGTGCCTGGAAAAGAAGGCGA  
TCTGTGGCTCGCTGCATCGAGCGGGCTTTACCACTCAACCAATGGCGGCAGC  
AGTTGGTCTGCAATCACCGGCGTATCCTCCGCGGTGAACGTGGGATTTGGTA  
AGTCTGCGCCCGGGTCGTCATACCCAGCCGTCTTTGTCGTCGGCACGATCGGA  
GGCGTTACGGGGGCGTACCGCTCCGACGACGGTGGGACGACCTGGGTACGG  
ATCAATGATGACCAGCACCAATACGGAAATTGGGGACAAGCAATCACCGGTG  
ACCCGCGAATTTACGGGCGGGTGTACATAGGCACGAACGGCCGTGGAATTGT  
CTACGGGGACATTGGTGGTGCGCCGTCCGGATCG

## FIGURE 9

Amino acid sequence of *Acidothermus cellulolyticus* 74 catalytic domain (741 amino acids)

ATTQPYTWSNVAIGGGGFVDGIVFNEGAPGILYVRTDIGGMYRWDAANGRWIPL  
LDWVGWNNWGYNGVVSAADPINTNKVWAAVGMYTNSWDPNDGAILRSSDQ  
GATWQITPLPFKLGGNMPGRGMGERLAVDPNNDNILYFGAPSGKGLWRSTDG  
ATWSQMTNFPDVGTYIANPTDTTGYQSDIQGVVWVAFDKSSSSLGQASKTIFVG  
VADPNNPVFWSRDGGATWQAVPGAPTGFIPHKGVFDPVNHVLYIATSNTGGPY  
DGSSGDVWKFSVTSGTWTRISPVPSTDTANDYFGYSGLTIDRQHPNTIMVATQIS  
WWPDTHIFRSTDGGATWTRIWDWTSYPNRSRLRYVLDISAEPWLTFGVQPNPPVPS  
PKLGWMDEAMAIDPFNSDRMLYGTGATLYATNDLTKWDSGGQIHAPMVKGLE  
ETA VNDLISPPSGAPLISALGDLGGFTHADVTAVPSTIFTSPVFTTGTSVDY AELNP  
SIIVRAGSFDPPSSQPNDRHVAFSTDGGKNWFQGSEPPGGVTTGGTVAASADGSRFV  
WAPGDPGQPVVYAVGFGNSWAASQGV PANAQIRSDRVNPKTFYALSNGTFYRS  
TDGGVTFQPVAAGLPSSGAVGVMFHAVPGKEGDLWLAASSGLYHSTNGGSSWS  
AITGVSSAVNVGFGKSAPGSSYPAVFVVG TIGGVTGAYRSDDGGTTWVRINDDQ  
HQYGNWGQAITGDPRIYGRVYIGTNGRGIVYGDIGGAPSGS

**Figure 10**

DNA sequence of *Thermobifida fusca* E3 (TfE3) cellulase including the cellulose binding domain - linker - catalytic domain but lacking a TfE3 signal sequence. (1677 bases)

```
GCCGGCTGCTCGGTGGACTACACGGTCAACTCCTGGGGTACCGGGTTCACCG
CCAACGTCACCATCACCAACCTCGGCAGTGCATCAACGGCTGGACCCTGGA
GTGGGACTTCCCCGGCAACCAGCAGGTGACCAACCTGTGGAACGGGACCTAC
ACCCAGTCCGGGCAGCACGTGTCGGTCAGCAACGCCCCGTACAACGCCTCCA
TCCCGGCCAACGGAACGGTTGAGTTCGGGTTC AACGGCTCCTACTCGGGCAG
CAACGACATCCCCTCCTTCAAGCTGAACGGGGTTACCTGCGACGGCTCG
GACGACCCCGACCCGAGCCAGCCCCTCCCCAGCCCTTCCCCAGCCCCA
CAGACCCGGATGAGCCGGGCGGCCGACCAACCCGCCCACCAACCCCGGCG
AGAAGGTCGACAACCCGTTTCGAGGGCGCCAAGCTGTACGTGAACCCGGTCTG
GTCGGCCAAGGCCGCGCTGAGCCGGGCGGTTCCGCGGTCGCCAACGAGTCC
ACCGCTGTCTGGCTGGACCGTATCGGCGCCATCGAGGGCAACGACAGCCCGA
CCACCGGCTCCATGGGTCTGCGCGACCACCTGGAGGAGGCCGTCCGCCAGTC
CGGTGGCGACCCGCTGACCATCCAGGTCGTCATCTACAACCTGCCCGGCCGC
GACTGCGCCGCGCTGGCCTCCAACGGTGAGCTGGGTCCCGATGAACTCGACC
GCTACAAGAGCGAGTACATCGACCCGATCGCCGACATCATGTGGGACTTCGC
AGACTACGAGAACCTGCGGATCGTCGCCATCATCGAGATCGACTCCCTGCCC
AACCTCGTCACCAACGTGGGCGGGAACGGCGGCACCGAGCTCTGCGCCTACA
TGAAGCAGAACGGCGGCTACGTCAACGGTGTCTGGCTACGCCCTCCGCAAGCT
GGGCGAGATCCCGAACGTCTACAACTACATCGACGCCGCCACCACGGCTGG
ATCGGCTGGGACTCCAACTTCGGCCCCCTCGGTGGACATCTTCTACGAGGCCG
CCAACGCCTCCGGCTCCACCGTGGACTACGTGCACGGCTTCATCTCCAACAC
GGCCAACTACTCGGCCACTGTGGAGCCGTACCTGGACGTCAACGGCACCGTT
AACGGCCAGCTCATCCGCCAGTCCAAGTGGGTGACTGGAACCAAGTACGTCTG
ACGAGCTCTCCTTCGTCCAGGACCTGCGTCAGGCCCTGATCGCCAAGGGCTTC
CGGTCCGACATCGGTATGCTCATCGACACCTCCCGCAACGGCTGGGGTGGCC
CGAACCGTCCGACCGGACCGAGCTCCTCCACCGACCTCAACACCTACGTTGA
CGAGAGCCGTATCGACCGCCGTATCCACCCCGGTA ACTGGTGCAACCAAGGCC
GGTGCGGGCCTCGGCGAGCGGCCACGGTCAACCCGGCTCCCGGTGTTGACG
CCTACGTCTGGGTGAAGCCCCCGGGTGAGTCCGACGGCGCCAGCGAGGAGAT
CCCGAACGACGAGGGCAAGGGCTTCGACCGCATGTGCGACCCGACCTACCAG
GGCAACGCCCCGCAACGGCAACAACCCCTCGGGTGCGCTGCCCAACGCCCCCA
TCTCCGGCCACTGGTTCTCTGCCAGTTCCGCGAGCTGCTGGCCAACGCCTAC
CCGCCTCTGTAA
```

**Figure 11**

Amino acid sequence of the *Thermobifida fusca* E3 - cellulase including the cellulose binding domain - linker - catalytic domain but lacking the Tfe3 signal sequence. (558 amino acids)

AGCSVDYTVNSWGTGFTANVTITNLGSAINGWTLWDFPGNQQTNLWNGTYT  
QSGQHVSVSNAFYNASIPANGTVEFGFNGSYSGSNDIPSSFKNLNGVTCDGSDDPD  
PEPSPSPSPSPPTDPDEPGGPTNPPTNPGEKVDNPFEGAKLYVNPVWSAKAAAE  
GGSANESTAVWLDRIGAIEGNDSPPTGSMGLRDHLEEA VRQSGGDPLTIQVVI  
YNLPGRDCAALASNGELGPDELDYKSEYIDPLADIMWDFADYENLRIVAIIIDS  
LPNLVTNVGGNGGTELCA YMKQNGGYVNGVGYALRKLGEIPNVYNYIDAAHH  
GWIGWDSNFGPSVDIFYEAAANASGSTVDYVHGFISNTANYSATVEPYLDVNGTV  
NGQLIRQSKWVDWNQYVDELSFVQDLRQALIAKGFRSDIGMLIDTSRNGWGGP  
NRPTGPSSSTDLNTYVDESRIIDRIHPGNWCNQAGAGLGERPTVNPAPGVDA YV  
WVKPPGESDGASEEIPNDEGKGFDRCMDPTYQGNARNGNNPSGALPNAPISGH  
WFSAQFRELLANAYPPL

**Figure 12**

DNA sequence of *Thermobifida fusca* E5 (TfE5) endoglucanase including the cellulose binding domain - linker and catalytic domain but lacking a TfE5 signal sequence. (1293 bases)

```
GCCGGTCTCACCGCCACAGTCACCAAAGAATCCTCGTGGGACAACGGCTACT
CCGCGTCCGTACCGTCCGCAACGACACCTCGAGCACCGTCTCCCAGTGGGA
GGTCGTCCTCACCCGTGCCCGGCGGCACTACAGTGGCCCAGGTGTGGAACGCC
CAGCACACCAGCAGCGGCAACTCCCACACCTTCACCGGGGTTTCCTGGAACA
GCACCATCCCCGCCCGGAGGCAACCGCCTCTTCCGGCTTCATCGCTTCCGGCAGC
GGCGAACCACCCACTGCACCATCAACGGCGCCCCCTGCGACGAAGGCTCCG
AGCCGGGCGGCCCCGGCGGTCCCGGAACCCCTCCCCGACCCCGGCACGCA
GCCCCGCACCGGCACCCCGGTTCGAGCGGTACGGCAAAGTCCAGGTCTGCGGC
ACCCAGTCTGCGACGAGCACGGCAAACCCGGTCCAACTGCGCGGCATGAGCA
CCCACGGCATCCAGTGGTTTCGACCACTGCCTGACCGACAGCTCGCTGGACGC
CCTGGCCTACGACTGGAAGGCCGACATCATCCGCCTGTCCATGTACATCCAG
GAAGACGGCTACGAGACCAACCCGCGCGGGCTTCACCGACCGGATGCACCAG
CTCATCGACATGGCCACGGCGCGCGGCCTGTACGTGATCGTGGACTGGCACA
TCCTCACCCCGGGCGATCCCCACTACAACCTGGACCGGGCCAAGACCTTCTTC
GCGGAAATCGCCCAGCGCCACGCCAGCAAGACCAACGTGCTCTACGAGATCG
CCAACGAACCCAACGGAGTGAGCTGGGCCTCCATCAAGAGCTACGCCGAAG
AGGTCATCCCGGTGATCCGCCAGCGCGACCCCGACTCGGTGATCATCGTGGG
CACCCGCGGTGTCGTCGCTCGGCGTCTCCGAAGGCTCCGGCCCCGCGGAG
ATCGCGGCCAACCCGGTCAACGCCTCCAACATCATGTACGCCTTCCACTTCTA
CGCGGCCTCGCACCGCGACAACCTCAACGCGCTGCGTGAGGCCTCCGAG
CTGTTCCCGGTCTTCGTACCGAGTTCGGCACCGAGACCTACACCGGTGACG
GCGCCAACGACTTCCAGATGGCCGACCGCTACATCGACCTGATGGCGGAACG
GAAGATCGGGTGGACCAAGTGGAATACTCGGACGACTTCCGTTCCGGCGCG
GTCTTCCAGCCGGGCACCTGCGCGTCCGGCGGCCCCGTGGAGCGGTTCGTGCG
TGAAGGCGTCCGGACAGTGGGTGCGGAGCAAGCTCCAGTCCTGA
```

**Figure 13**

Amino acid sequence of the *Thermobifida fusca* E5 -cellulase including the cellulose binding domain - linker - catalytic domain but lacking a Tfe5 signal sequence. (430 amino acids)

AGLTATVTKESSWDNGYSASVTVRNDTSSTVSQWEVVLTLPGGTTVAQVWNAQ  
HTSSGNSHTFTGVSWNSTIPPGGTASSGFIASGSGEPHCTINGAPCDEGSEPGGP  
GGPGTPSPDPGTQPGTGTPVERYGKVQVCGTQLCDEHGNPVQLRGMSTHGIQW  
FDHCLTDSSLDALAYDWKADIIRLSMYIQEDGYETNPRGFTDRMHQLIDMATAR  
GLYVIVDWHILTPGDPHYNLDRAKTFFAEIAQRHASKTNVLYEIANEPNGVSWA  
SIKSYAEEVIPVIRQRPDSVIIVGTRGWSSLGVSEGS GPAEIAANPVNASNIMYAF  
HFYAASHRDNYLNALREASELFPVFEFGTETTYTGDGANDFQMADRYIDLMA  
ERKIGWTKWNYSDDFRSGAVFQPGTCASGGPWSSSLKASGQWVRSLQS

Figure 14

DNA sequence of CBH1-E1 fusion (2656 bases)

*T.reesei cbh1* signal sequence+catalytic domain+linker+added amino acidsSKR+*Acidothermus cellulolyticus* GH5A catalytic domain

ATGTATCGGAAGTTGGCCGTCATCTCGGCCTTCTTGGCCACAGCTCGTGCTCA  
GTCGGCCTGCACTCTCCAATCGGAGACTCACCCGCCTCTGACATGGCAGAAA  
TGCTCGTCTGGTGGCACTTGCACTCAACAGACAGGCTCCGTGGTCATCGACG  
CCAAGTGGCGCTGGACTCACGCTACGAACAGCAGCACGAAGTGTACGATGG  
CAACACTTGAGCTCGACCCTATGTCCTGACAACGAGACCTGCGCGAAGAAC  
TGCTGTCTGGACGGTGGCGCTACGCGTCCACGTACGGAGTTACCACGAGCG  
GTAACAGCCTCTCCATTGGCTTTGTACCCAGTCTGCGCAGAAGAACGTTGGC  
GCTCGCCTTTACCTTATGGCGAGCGACACGACCTACCAGGAATTCACCCTGCT  
TGGCAACGAGTTCTCTTTCGATGTTGATGTTTCGCAGCTGCCGTAAGTGACTT  
ACCATGAACCCCTGACGTATCTTCTTGTGGGCTCCCAGCTGACTGGCCAATTT  
AAGGTGCGGCTTGAACGGAGCTCTCTACTTCGTGTCCATGGACGCGGATGGT  
GGCGTGAGCAAGTATCCCAACACACCGCTGGCGCCAAGTACGGCACGGGGT  
ACTGTGACAGCCAGTGTCCCCGCGATCTGAAGTTCATCAATGGCCAGGCCAA  
CGTTGAGGGCTGGGAGCCGTCATCCAACAACGCAAACACGGGCATTGGAGG  
ACACGGAAGCTGCTGCTCTGAGATGGATATCTGGGAGGCCAACTCCATCTCC  
GAGGCTCTTACCCCCCACCCTTGCACGACTGTGCGGCCAGGAGATCTGCGAGG  
GTGATGGGTGCGGCGGAACCTTACTCCGATAACAGATATGGCGGCACTTGCGA  
TCCCCGATGGCTGCGACTGGAACCCATACCGCCTGGGCAACACCAGCTTCTAC  
GGCCCTGGCTCAAGCTTTACCCTCGATACCACCAAGAAATTGACCGTTGTCAC  
CCAGTTCGAGACGTCGGGTGCCATCAACCGATACTATGTCCAGAATGGCGTC  
ACTTTCCAGCAGCCCAACGCCGAGCTTGGTAGTTACTCTGGCAACGAGCTCA  
ACGATGATTACTGCACAGCTGAGGAGGCAGAATTCGGCGGATCCTCTTTCTC  
AGACAAGGGCGGCCTGACTCAGTTCAAGAAGGCTACCTCTGGCGGCATGGTT  
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GACAAAGAGTCAAGCAGCTGACTGAGATGTTACAGTACTACGCCAACATGCT  
GTGGCTGGACTCCACCTACCCGACAAACGAGACCTCCTCCACACCCGGTGCC  
GTGCGCGGAAGCTGCTCCACCAGCTCCGGTGTCCCTGCTCAGGTCTGAATCTC  
AGTCTCCCAACGCCAAGGTCACCTTCTCCAACATCAAGTTCGGACCCATTGGC  
AGCACCGGCAACCCTAGCGGCGGCAACCCTCCCGGCGGAAACCCGCCTGGCA  
CCACCACCACCCGCCGCCAGCCACTACCACTGGAAGCTCTCCCGGACCTAC  
TAGTAAGCGGGCGGGCGGGCGGCTATTGGCACACGAGCGGCCGGGAGATCCT  
GGACGCGAACAACGTGCCGGTACGGATCGCCGGCATCAACTGGTTTGGGTTT  
GAAACCTGCAATTACGTCTGTGACGGTCTCTGGTCACGCGACTACCGCAGCA  
TGCTCGACCAGATAAAGTCGCTCGGCTACAACACAATCCGGCTGCCGTACTC  
TGACGACATTCTCAAGCCGGGCACCATGCCGAACAGCATCAATTTTACCAG  
ATGAATCAGGACCTGCAGGGTCTGACGTCCTTGACAGGTCATGGACAAAATCG  
TCGCGTACGCCGGTCAGATCGGCCTGCGCATCATTCTTGACCGCCACCGACC  
GGATTGCAGCGGGCAGTCGGCGCTGTGGTACACGAGCAGCGTCTCGGAGGCT  
ACGTGGATTTCGACCTGCAAGCGCTGGCGCAGCGCTACAAGGGAAACCCGA

CGGTCGTCGGCTTTGACTTGCACAACGAGCCGCATGACCCGGCCTGCTGGGG  
CTGCGGCGATCCGAGCATCGACTGGCGATTGGCCGCCGAGCGGGCCGAAAC  
GCCGTGCTCTCGGTGAATCCGAACCTGCTCATTTTCGTCGAAGGTGTGCAGAG  
CTACAACGGAGACTCCTACTGGTGGGGCGGCAACCTGCAAGGAGCCGGCCA  
GTACCCGGTCGTGCTGAACGTGCCGAACCGCCTGGTGTACTCGGCGCACGAC  
TACGCGACGAGCGTCTACCCGCAGACGTGGTTCAGCGATCCGACCTTCCCCA  
ACAACATGCCCCGGCATCTGGAACAAGAACTGGGGATACCTCTTCAATCAGAA  
CATTGCACCGGTATGGCTGGGCGAATTCGGTACGACACTGCAATCCACGACC  
GACCAGACGTGGCTGAAGACGCTCGTCCAGTACCTACGGCCGACCGCGCAAT  
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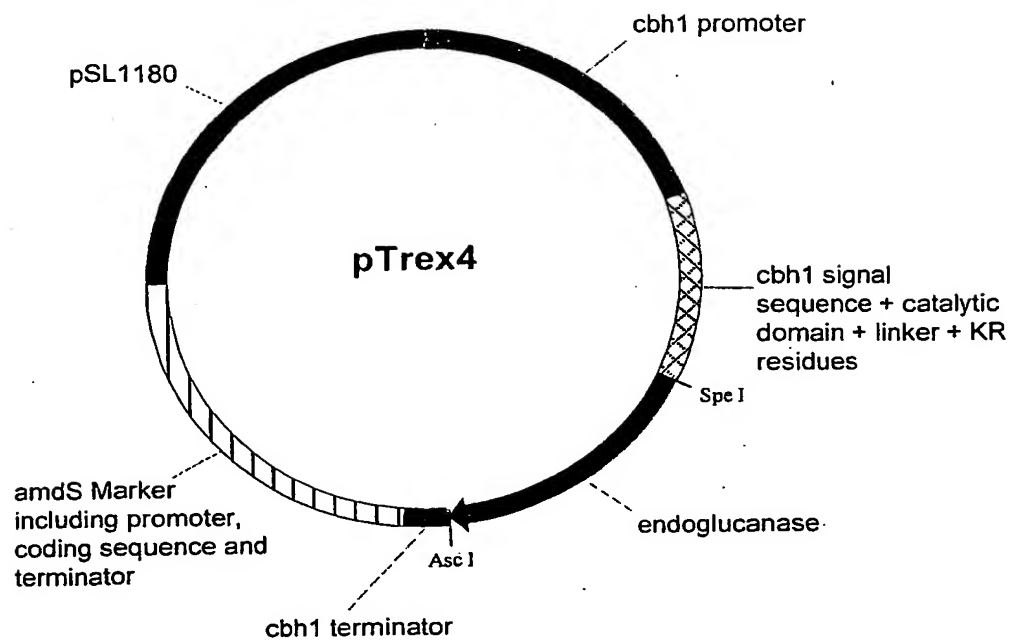


**Figure 15**

Amino acid sequence of CBH1-E1 fusion (841 amino acids)  
*Treesei cbh1* signal sequence+catalytic domain+linker+added amino acids  
SKR+*Acidothermus cellulolyticus* GH5A catalytic domain

MYRKLAVISAFLATARAQSACTLQSETHPPLTWQKCSSGGTCTQQTGSVVIDAN  
WRWTHATNSSTNCYDGNTWSSTLCPDNETCAKNCCLDGAAYASTYGVTTSGNS  
LSIGFVTQSAQKNVGARLYLMASDTTYQEFTLLGNEFSFDVDVSQLPCGLNGAL  
YFVSMDADGGVSKYPTNTAGAKYGTGYCDSQCPRDLKFINGQANVEGWEPSSN  
NANTGIGGHGSCCSEMDIWEANSISEALTPHPCTTVGQEICEGDGCGGTYSNRY  
GGTCDPDGCDWNPYRLGNTSFYGPSSFTLDTTKKLTVVVTQFETSGAINRYVQ  
NGVTFQQPNAELGSYSGNELNDDYCTAEAEFGGSSFSKGGGLTQFKKATSGGM  
VLVMSLWDDYYANMLWLDSTYPTNETSSTPGAVRGSCSTSSGVPQVESQSPN  
AKVTFSNIKFGPIGSTGNPSGGNPPGGNPPGTTTTTRRPATTTGSSPGPTSKRAGG  
YWHTSGREILDANNVPVRIAGINWFGFETCNVYVHGLWSRDYRSMLDQIKSLGY  
NTIRLPYSDDILKPGTMPNSINFYQMNQDLQGLTSLQVMDKIVAYAGQIGLRILD  
RHRPDCSGQSALWYTSSVSEATWISDLQALAARYKGNPTVVGFDLHNEPHDPAC  
WCGGDPSIDWRLAAERAGNAVLSVNPNNLLIFVEGVQSYNGDSYWWGGNLQGA  
GQYPVVLNVPNRLVYSAHDYATSVYPQTFWSDPTFPNNMPGIWNKNWGYLFN  
QNIAPVWLGEFGTTLQSTTDQTLWKLTVQYLRPTAQYGADSFQWTFWSWNPDS  
GDTGGILKDDWQTVDTVKDGYLAPIKSSIFDPVG

Figure 16



## Figure 17

DNA sequence of pTrex4 (10239 bases)

AAGCTTAACTAGTACTTCTCGAGCTCTGTACATGTCCGGTCGCGACGTACGCG  
TATCGATGGCGCCAGCTGCAGGCGGCCGCCTGCAGCCACTTGCA GTCCCGTG  
GAATTCTCACGGTGAATGTAGGCCTTTTGTAGGGTAGGAATTGTCACTCAAGC  
ACCCCCAACCTCCATTACGCCTCCCCCATAGAGTTCCCAATCAGTGAGTCATG  
GCACTGTTCTCAAATAGATTGGGGGAGAAGTTGACTTCCGCCCAGAGCTGAAG  
GTCGCACAACCGCATGATATAGGGTCGGCAACGGCAAAAAAGCACGTGGCT  
CACCGAAAAGCAAGATGTTTGCGATCTAACATCCAGGAACCTGGATACATCC  
ATCATCACGCACGACCACTTTGATCTGCTGGTAAACTCGTATTCGCCCTAAAC  
CGAAGTGACGTGGTAAATCTACACGTGGGCCCTTTTCGGTATACTGCGTGTGT  
CTTCTCTAGGTGCCATTCTTTTCCCTTCCTCTAGTGTTGAATTGTTTGTGTTGG  
AGTCCGAGCTGTAACCTCTGAATCTCTGGAGAATGGTGGACTAACGACT  
ACCGTGACCTGCATCATGTATATAATAGTGATCCTGAGAAGGGGGGTTTGG  
AGCAATGTGGGACTTTGATGGTCATCAAACAAAGAACGAAGACGCCTCTTTT  
GCAAAGTTTTGTTTCGGCTACGGTGAAGAACTGGATACTTGTTGTGTCTTCTG  
TGTATTTTTGTGGCAACAAGAGGCCAGAGACAATCTATTCAAACACCAAGCT  
TGCTCTTTTGAGCTACAAGAACCTGTGGGGTATATATCTAGAGTTGTGAAGTC  
GGTAATCCCGCTGTATAGTAATACGAGTCGCATCTAAATACTCCGAAGCTGCT  
GCGAACCCGGAGAATCGAGATGTGCTGGAAAGCTTCTAGCGAGCGGCTAAAT  
TAGCATGAAAGGCTATGAGAAATTCTGGAGACGGCTTGTTGAATCATGGCGT  
TCCATTCTTCGACAAGCAAAGCGTTCCGTCGCAGTAGCAGGCACTCATTCCCG  
AAAAAACTCGGAGATTCCTAAGTAGCGATGGAACCGGAATAATATAATAGGC  
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TACCCGTACAAGTCGTAATCACTATTAACCCAGACTGACCGGACGTGTTTTGC  
CCTTCATTTGGAGAAATAATGTCATTGCGATGTGTAATTTGCCTGCTTGACCG  
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TGA CTCGGAGCGTTTTGCATACAACCAAGGGCAGTGATGGAAGACAGTGAAA  
TGTTGACATTCAAGGAGTATTTAGCCAGGGATGCTTGAGTGTATCGTGTAAG  
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TGCCTAAGATCTCGGGCCCTCGGGCCTTCGGCCTTTGGGTGTACATGTTTGTG  
CTCCGGGCAAATGCAAAGTGTGGTAGGATCGAACACACTGCTGCCTTTACCA  
AGCAGCTGAGGGTATGTGATAGGCAAATGTTCAAGGGGCCACTGCATGGTTTC  
GAATAGAAAGAGAAGCTTAGCCAAGAACAATAGCCGATAAAGATAGCCTCA  
TTAAACGGAATGAGCTAGTAGGCAAAGTCAGCGAATGTGTATATATAAAGGT  
TCGAGGTCCGTGCCTCCCTCATGCTCTCCCATCTACTCATCAACTCAGATCC  
TCCAGGAGACTTGTACACCATCTTTTGAGGCACAGAAACCCAATAGTCAACC  
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ACTGCTACGATGGCAACACTTGGAGCTCGACCCTATGTCCTGACAACGAGAC  
CTGCGGAAGAAGTGTGTCTGGACGGTGCCGCCTACGCGTCCACGTACGGA  
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GAAGAACGTTGGCGCTCGCCTTTACCTTATGGCGAGCGACACGACCTACCAG  
GAATTCACCCTGCTTGGCAACGAGTTCTCTTTTCGATGTTGATGTTTCGCAGCT  
GCCGTAAGTGA CTTACCATGAACCCCTGACGTATCTTCTTGTGGGCTCCCAGC  
TGA CTGGCCAATTTAAGGTGCGGCTTGAACGGAGCTCTCTACTTCGTGTCCAT  
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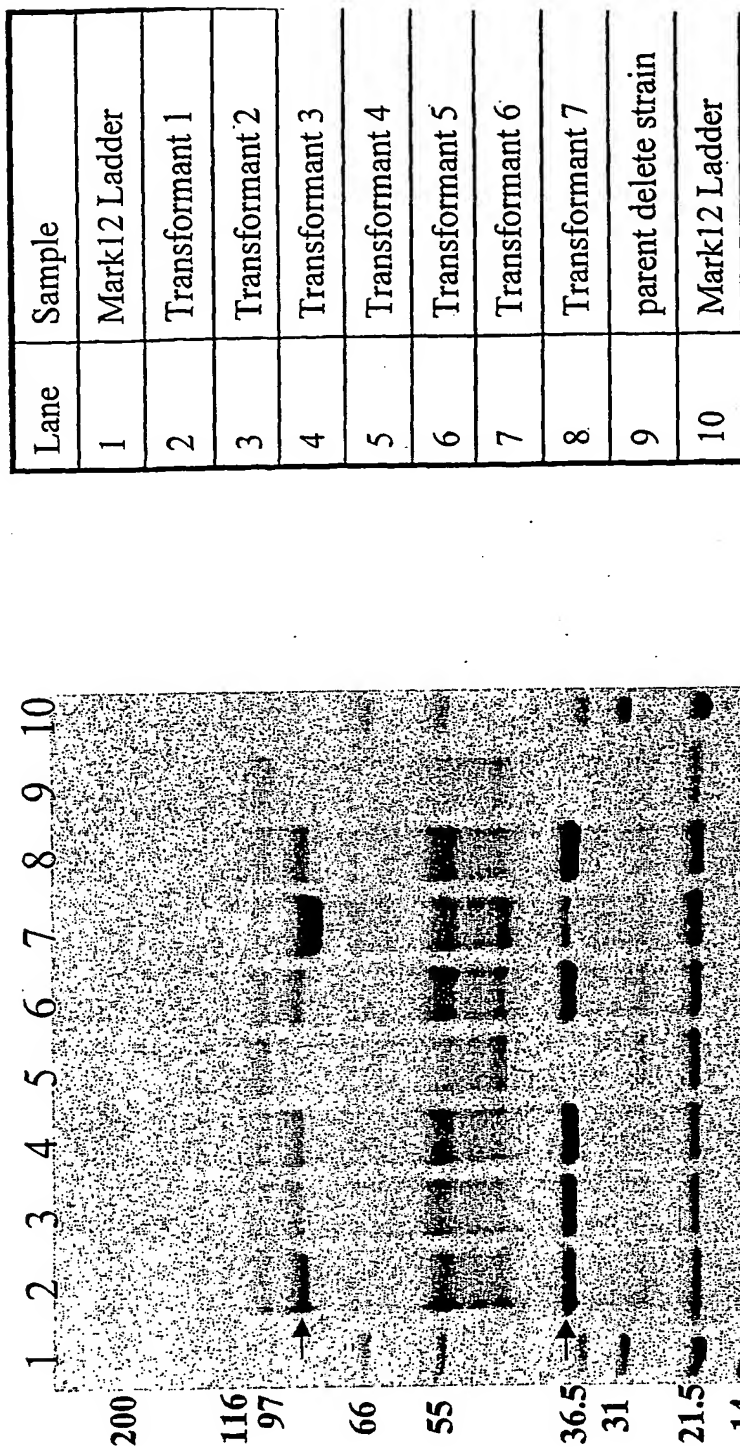
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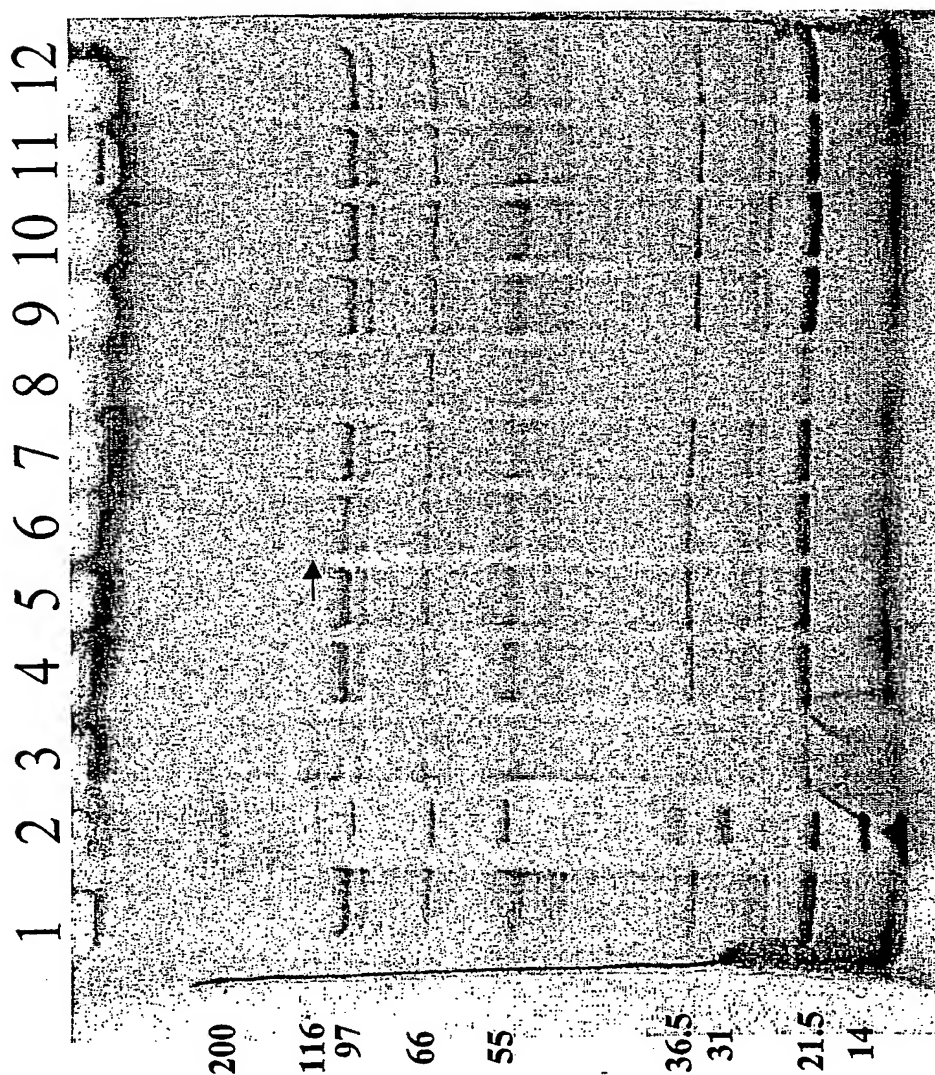
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TTCCAGTCACGACGTTGTAAACGACGGCCAGTGCC

Figure 18  
 SDS-PAGE gel of supernate samples of shake flask grown *T.reesei* delete strain transformed with the fusion expression construct *cbh1-E1*



The fusion protein is indicated by the upper arrow, the cleaved E1 catalytic domain is indicated by the lower arrow

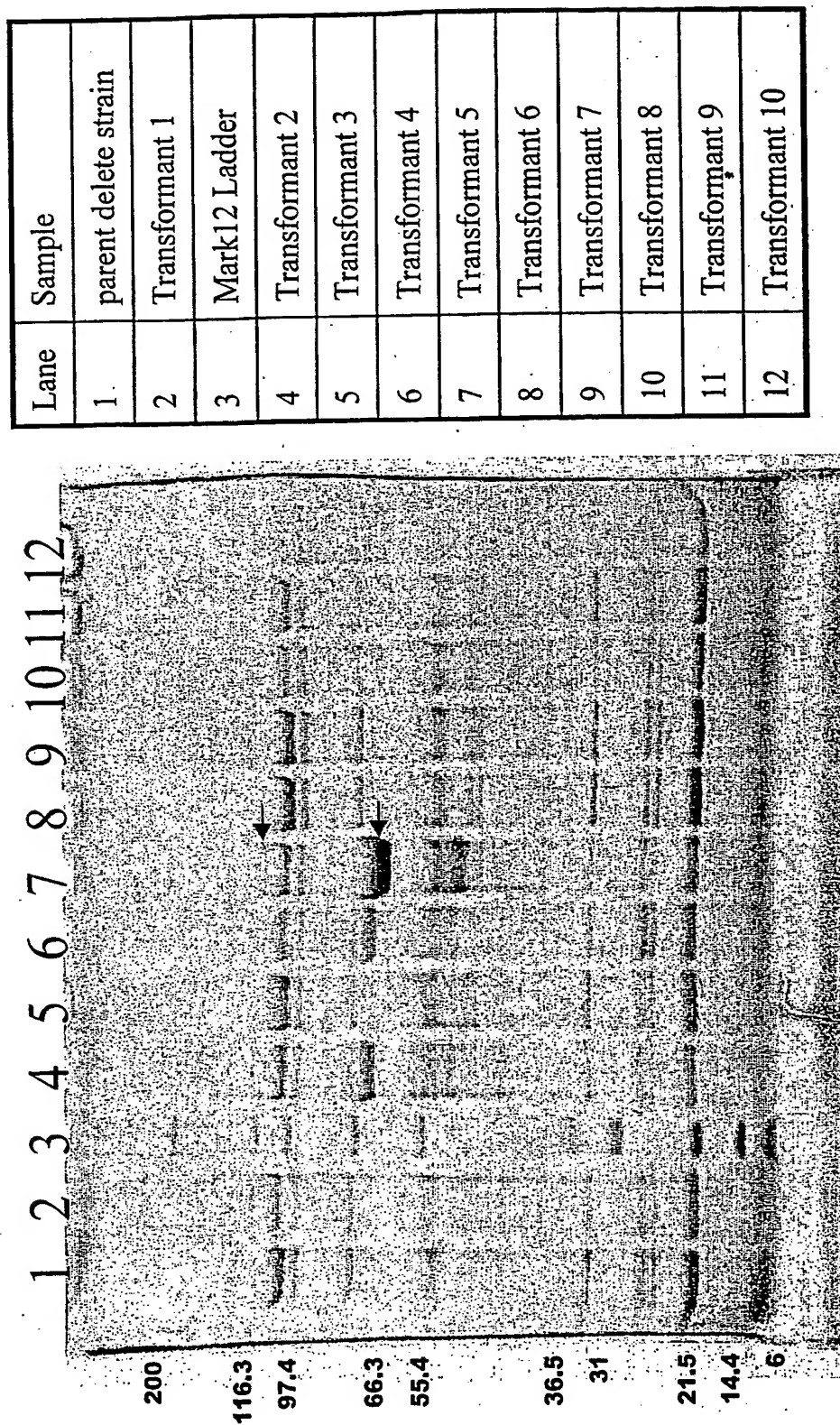
Figure 19  
SDS-PAGE gel of supernate samples of shake flask grown *T.reesei* delete strain transformed with the fusion expression construct *cbh1-GH48*



Lane	Sample
1	parent delete strain
2	Mark12 Ladder
3	Transformant 1
4	Transformant 2
5	Transformant 3
6	Transformant 4
7	Transformant 5
8	Transformant 6
9	Transformant 7
10	Transformant 8
11	Transformant 9
12	Transformant 10

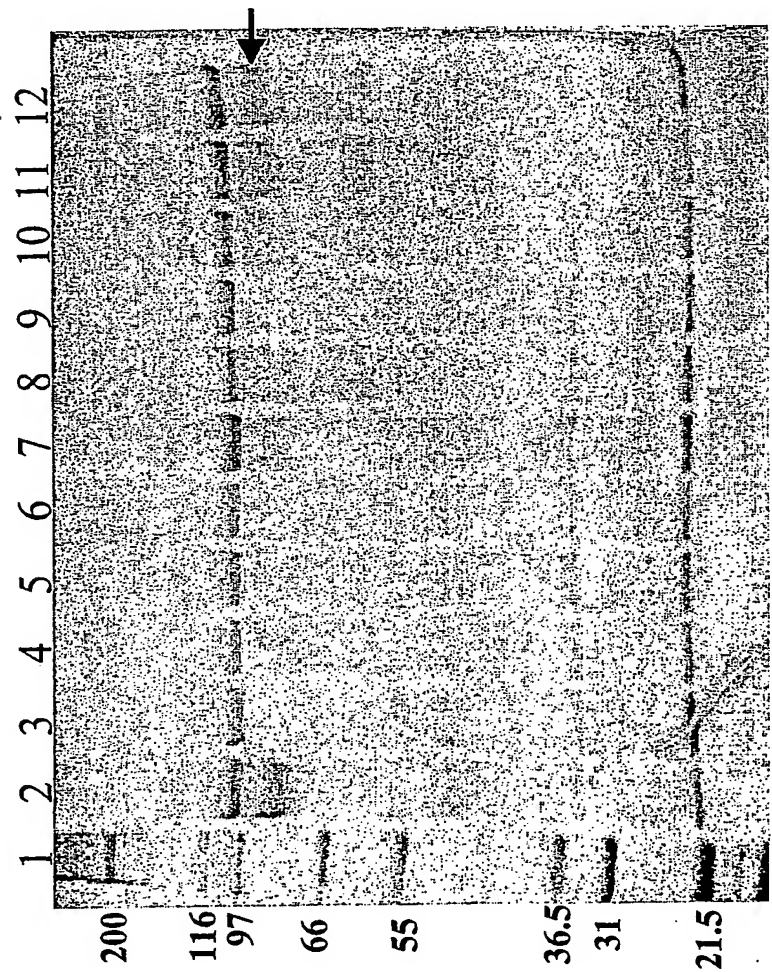
The fusion protein is indicated by the arrow

Figure 20  
SDS-PAGE gel of supernate samples of shake flask grown *T. reesei* delete strain transformed with the fusion expression construct *cbh1-GH74*



The fusion protein is indicated by the upper arrow, the cleaved GH74 catalytic domain is indicated by the lower arrow

Figure 21  
SDS-PAGE gel of supernate samples of shake flask grown *T.reesei* delete strain transformed with the fusion expression construct *cbh1-E3*

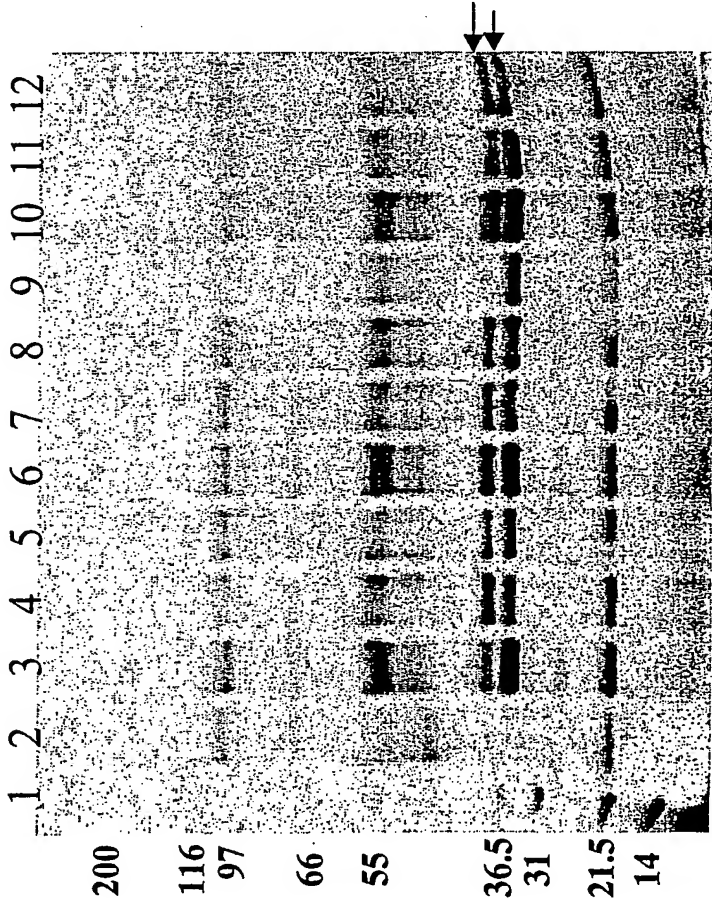


arrow indicates new protein expressed  
in *cbh1-E3* transformants

Lane	Sample
1	Mark12 Ladder
2	Transformant 1
3	Transformant 2
4	Transformant 3
5	Transformant 4
6	Transformant 5
7	Transformant 6
8	Transformant 7
9	Transformant 8
10	Transformant 9
11	Transformant 10
12	Transformant 11

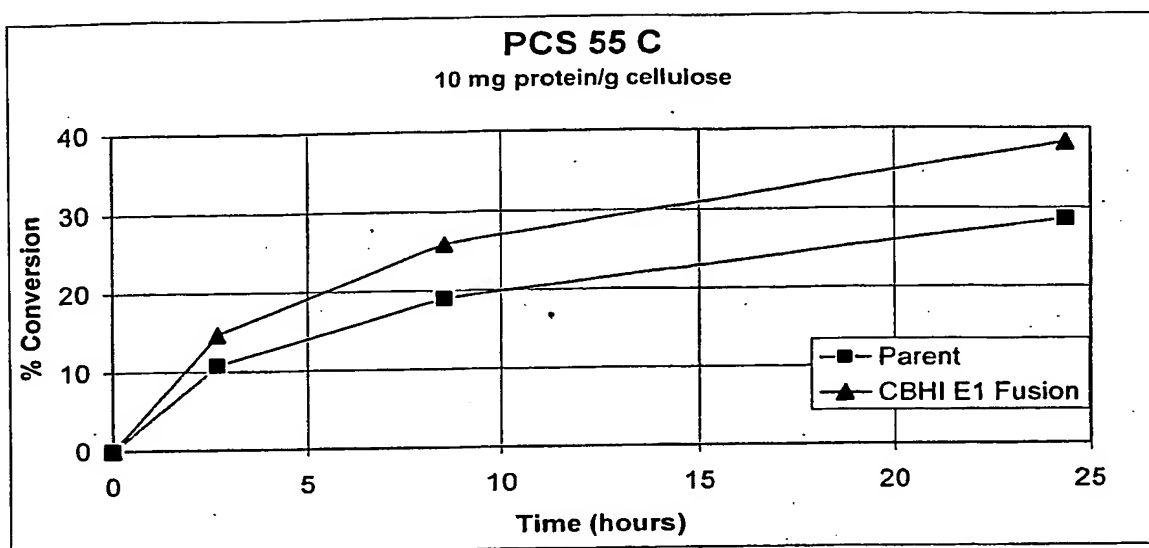
Figure 22  
SDS-PAGE gel of supernate samples of shake flask grown *T.reesei* delete strain transformed with the fusion expression construct cbh1-E5

Lane	Sample
1	Mark12 Ladder
2	parent delete strain
3	Transformant 1
4	Transformant 2
5	Transformant 3
6	Transformant 4
7	Transformant 5
8	Transformant 6
9	Transformant 7
10	Transformant 8
11	Transformant 9
12	Transformant 10



E5 is indicated by the lower arrows

FIGURE 23



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